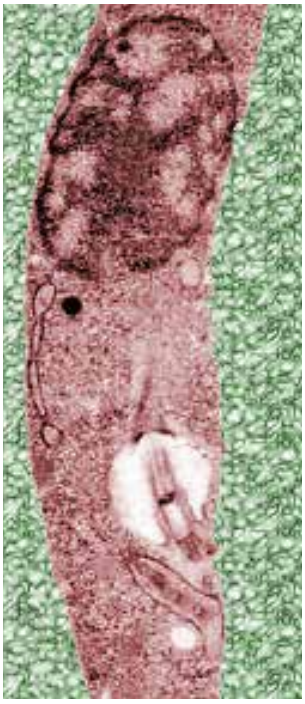


ILRI RESEARCH BRIEF 31
November 2014

# Forty years of ILRI's immunology research: An impact narrative

## Did you know?

- The investment made at ILRAD/ILRI to develop vaccines for AAT and ECF has been less than 1% of that invested in HIV and malaria research. However, ILRAD/ILRI's research has made a significant contribution to immunological research worldwide.
- Development of murine (rodent) monoclonal antibodies against bovine immune system biomarkers was a key milestone in ILRAD's immunological research and in contributing to ruminant immunology worldwide.
- Understanding how virus infected cells are killed proved transformative in the understanding of how immune responses control T. parva infected cells and thus ECF.
- Ndama, a West African breed of cattle which is moderately tolerant of trypanosomaisis, were imported to ILRAD/ILRI as embryos from West Africa and implanted in cows in Kenya which served as surrogate mothers. The ILRAD/ILRI Ndama herd provided investigators with the opportunity to study mechanisms of trypanosomiasis resistance.
- ILRAD/ILRI has developed diagnostic tests for five major African tick-borne diseases of cattle.
- ILRI is adopting genomics based approaches to immunology, often described as "reverse" immunology, to tease apart both antibody and T-cell responses.

Since 1973, veterinary immunology has been a key aspect of research at ILRI and its predecessor, the International Laboratory for Research on Animal Diseases (ILRAD). ILRAD was specifically established to undertake basic research to develop effective and economic control measures for livestock diseases that seriously constrain global food production. As part of this mandate, ILRAD focused "initially on intensive research concerning the immunological and related aspects of controlling trypanosomiasis and theileriosis (mainly East Coast fever) …", with the goal of decreasing the incidence and/or severity of disease. Over the years, cutting edge tools, technologies and approaches have been implemented to increase understanding of the bovine immune system and mechanisms of pathogen control. Despite limited funding, compared to human immunological research, ILRAD/ILRI have made tremendous progress in bovine immunological research which remains a research focus at ILRI.

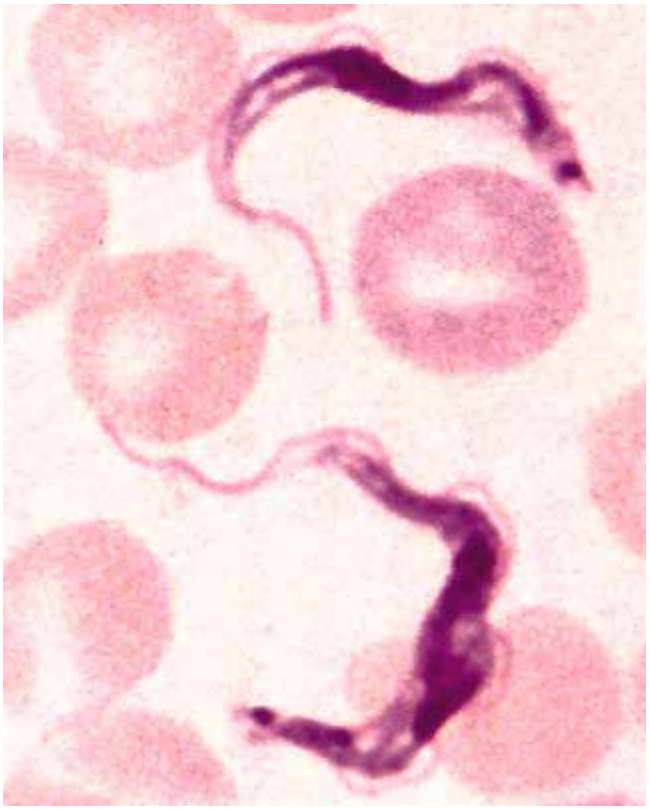
## Context:

Animal African Trypanosomiasis (AAT) and East Coast fever (ECF) are two diseases that severely constrain livestock production in sub-Saharan Africa. AAT is caused by trypanosome protozoans that live in blood and tissue fluids and are transmitted by tsetse flies. The disease prevents cattle ranching and mixed crop-livestock agriculture in vast areas across Africa (about 10 million km2), where tsetse and trypanosomiasis challenge is high. Cattle-based agriculture can be practiced on the fringe of the tsetse

habitat, where trypanosome challenge is less, supported by chemotherapy and tsetse control. Yet despite these interventions, AAT results in a loss of US$ 4 billion/yr in productivity.

ECF is caused by Theileria parva, another parasitic protozoan, which lives in the lymphocytes (white blood cells) and red blood cells of its mammalian host. ECF occurs in 11 countries in eastern, southern and central Africa and kills over 1 million cattle every year; annual economic losses as a result of ECF are valued at over US$300 million.

*Trypanosoma brucei*



## Understanding livestock immunity

When ILRAD's research and support facilities were inaugurated in 1978, it was known from studies in mice and humans that lymphocytes (white blood cells that play a leading role in mammalian immune systems) could be divided into two groups: B cells and T cells. B cells primarily tackle extra-cellular disease-causing organisms (e.g. AAT) while T cells primarily tackle intra-cellular pathogens, (e,g. ECF). B cells create antibodies in the presence of a particular sub-component, or 'antigen', of the pathogen. T cells are divided into sub-groups which have specific roles. For example, cytotoxic (CD8) T lymphocytes destroy infected cells, whilst T helper lymphocytes (CD4) send 'help' signals to B cells and CD8 cytotoxic cells, and can themselves play a direct role in immunity to some pathogens. Often, all of these immune responses may work together to clear infections (e.g. ECF).

Immune systems in mammals have evolved to protect them against infection by pathogens and to initiate a rapid response if these agents are re-encountered; a response that is exploited by vaccination. Research at ILRAD/ILRI focused on African trypanosome and Theileria strain diversity, the nature of protective immune responses against these pathogens, and the identify of parasite components (putative vaccine antigens) that elicit protective immune responses.

"The monoclonal antibodies produced at ILRAD and its early work with domestic animal infectious diseases set a standard of excellence for the world community working on infectious diseases of domestic animals, especially cattle."
Gary Splitter, Professor of Veterinary Immunology, University of Wisconsin, USA

## Understanding Theileria parva

Infective Theileria particles (sporozoites) are spread by ticks. Once in the body, they invade host lymphocytes and cause them to multiply in an uncontrolled manner like a severe cancer of the immune system, which kills infected cattle in a few weeks.

To support its research on mechanisms of infection and control, ILRAD recruited staff with expertise in what were then cutting edge immunology technologies. These techniques included the development of monoclonal antibodies, which are specific to a single antigen and produced by a single B cell and its progeny, and the generation of T cell clones that retain the specificity and function of their precursor T cell. The first ILRAD monoclonal antibodies were produced against trypanosome and theilerial antigens in the late 1970s, while the first T cell clones were produced in the mid 1980s.

Furthermore, monoclonal antibodies were identified that prevented infection of cattle lymphocytes by Theileria sporozoites. These were found to react with a sporozoite coat protein called p67. The T. parva gene encoding this protein has been identified, cloned and expressed in bacteria providing material for ECF vaccine trials, which are ongoing. Similarly, Theileria genes that encode antigens that serve as targets for CD8 T cells, which play a role in immunity to ECF, have also been identified.

It was first shown that CD8 T cells kill Theileria parva infected cells in vitro and upon transfer from immune cattle into infected naïve twins. These findings have thus focused research on antigens targeted by both antibodies and CD8 T cells for inclusion in an ECF vaccine.

## Understanding tryps

African trypanosomes are transferred into mammals in the saliva of tsetse flies. The parasites invade, live and multiply in the blood plasma and tissue fluids of infected mammals. In trypanosomiasis susceptible hosts, including cattle and goats, the parasites cause chronic infections.

Clearance of African trypanosomes from the infected host by the immune system is compromised due to variation of their glycoprotein coat (known as variable surface

glycoprotein - VSG). The vast number of antigenic variants of the trypanosome VSG, and the absence of conserved antigens on the trypanosome surface, prevented the ILRAD immunology team from developing a conventional vaccine that protects against infection with the parasites.

The team turned their attention to trypanotolerance mechanisms in Cape buffalo, N'dama cattle and certain strains of inbred mice to determine ways to reduce the severity of trypanosomiasis-associated immunopathology in susceptible Boran cattle. Although the studies did not definitely identify resistance genes for Boran, they left a legacy of infection-induced immune response data. Furthermore, the investigatory team made some notable contributions to understanding trypanosome biology and immunology and contributed to immune response analysis of other antigenically variable parasites (see Shapiro quote)

"The challenge of trying to make a vaccine against African trypanosomes with their variable surface antigens prepared me to think about the challenges of making a vaccine against the extremely variable human immunodeficiency virus on which I now work."
Stuart Shapiro, Vaccine Research Program, National Institute of Health, USA

In addition, immune response analyses in trypanosome infected AAT susceptible Boran cattle and AAT tolerant N'Dama cattle carried out at ILRAD/ILRI support the view that an appropriate T helper cell (CD4 T cell) response might facilitate host control of trypanosomiasis, a line of investigation that is currently being pursued by investigators outside of ILRI.

## Providing global expertise

The tools, techniques and approaches developed by the ILRAD/ILRI immunological team are used worldwide and have facilitated in-depth immunological analyses of immune responses in cattle in general.

In addition, ILRAD/ILRI alumni hold senior scientific appointments at institutes throughout the world and continue their studies in veterinary immunology and parasitology. Whilst capacity building in this research area was not specifically a mandate of ILRAD/ILRI, there is no doubt that the expertise and facilities at ILRI have contributed to work on a wide range of diseases worldwide.

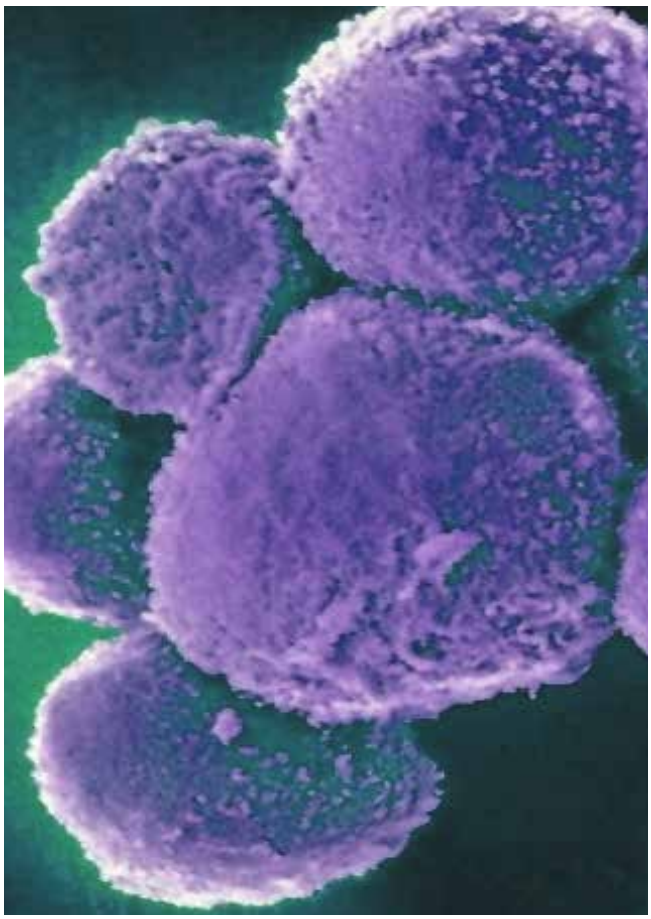
Top tier scientists recruited to ILRAD were very willing to establish collaborations with other scientists and share the reagents and protocols they produced. However, perhaps more important was that the critical mass of scientists at ILRAD in those early years generated very important questions regarding the mechanisms of how pathogens might cause disease. It was those questions that infused others beyond ILRAD with a scientific purpose."
Gary Splitter, Professor of Veterinary Immunology, University of Wisconsin, USA

There is also no doubt that over the years, the ILRAD/ILRI's immunology and immunoparasitology research resulted in significant impact with much being achieved on a moderate budget. Output from the research programs includes monoclonal antibodies specific for bovine leukocytes and immunoglobulins, which are being used in many laboratories throughout the world.

Whilst the immunology/immunoparasitology program has not affected the incidence of theileriosis or trypanosomiasis so far, it is envisaged that control for AAT and ECF will ultimately be achieved. It is particularly important to note that investment in the immunological control of these and other challenging diseases does not result in a quick fix, as is also evidenced by human immunological research into cancer, HIV/AIDS, and malaria (for which vaccines also do not exist). Nevertheless, immunological research at ILRI continues to focus on improving the 'infection and treatment' vaccine for ECF, as well as development of a recombinant subunit vaccine, and more recently on improving the vaccine for contagious bovine pleuropneumonia (CBPP) and peste des petits ruminants (PPR).

Whilst in recent years, ILRI has curtailed its immunological based studies on AAT, it continues a focus on identifying traits and genes in mice and cattle that determine susceptibility and tolerance to the disease, which will, it is expected, expedite development of East African breeds of cattle that remain productive under trypanosomiasis pressure.

*Natural Born Killer (cells)*



## References


Baldwin, C. L., A. J. Teale, J. G. Naessens, B. M. Goddeeris, N. D. MacHugh and W. I. Morrison. 1986. Characterization of a subset of bovine T lymphocytes that express BoT4 by monoclonal antibodies and function: similarity to lymphocytes defined by human T4 and murine L3T4. *Journal of Immunology* 136(12): 4385-4391.

Ellis, J. A., C. L. Baldwin, N. D. MacHugh, A. Bensaid, A. J. Teale, B. M. Goddeeris and W. I. Morrison. 1986. Characterization by a monoclonal antibody and functional analysis of a subset of bovine T lymphocytes that express BoT8, a molecule analogous to human CD8. *Immunology* 58(3): 351-358.

Graham SP, Pelle R, Yamage M, Mwangi DM, Honda Y, et al. (2008) Characterization of the fine specificity of bovine CD8 T-cell responses to defined antigens from the protozoan parasite Theileria parva. Infect Immun 76: 685-694

McKeever, D. J., E. L. Taracha, E. L. Innes, N. D. MacHugh, E. Awino, B. M. Goddeeris and W. I. Morrison. 1994. Adoptive transfer of immunity to Theileriaparva in the CD8+ fraction of responding efferent lymph. PNAS 91(5): 1959-1963

Musoke, A., Morzaria, S., Nkonge, C., Jones, E., and Nene, V. 1992. A recombinant sporozoite surface antigen of Theileriaparva induces protection in cattle. PNAS 89(2): 514-518.

Naessens, J., Teale, A.J., Sileghem, M. (2002. Identification of mechanisms of natural resistance to African trypanosomiasis in cattle. *Veterinary Immunology and Immunopathology* 87: 187-194.

Paling, R.W., Moloo, S.K., Scott, J.R., Gettinby, G., McOdimba, F.A., et al. 1991. Susceptibility of N'Dama and Boran cattle to sequential challenges with tsetse-transmitted clones of Trypanosoma congolense. *Parasite Immunology* 13: 427-445.


Photo credits:
Page 1: (left) AJ Cann (right) Jorge Almeida
Page 2: AJ Cann
Page 3: AJ Cann

This is one of a series of briefs documenting the impacts of ILRI's research.

It was prepared by WRENmedia with inputs by Samuel Black and Vish Nene.


*Contact:*
Vish Nene
Director, Vaccine Biosciences
ILRI, Kenya
ilri-vaccines@cgiar.org



ilri.org
*Better lives through livestock*
ILRI is a member of the CGIAR Consortium

Box 30709, Nairobi 00100, Kenya
Phone: + 254 20 422 3000
Fax: +254 20 422 3001
Email: ILRI-Kenya@cgiar.org

Box 5689, Addis Ababa, Ethiopia
Phone: +251 11 617 2000
Fax: +251 11 617 2001
Email: ILRI-Ethiopia@cgiar.org





November 2014